JOURNAL OF THE AMERICAN COLLEGE OF CARDIOLOGY
VOL. 64, NO. 3, 2014

ISSN 0735-1097/$36.00
PUBLISHED BY ELSEVIER INC.
http://dx.doi.org/10.1016/j.jacc.2014.04.045

EDITORIAL COMMENT

Single Nucleotide Variances Can Account for Loss of microRNA Function

The Emerging Cross Talk Between Genetics and Epigenetics*

Ren-Ke Li, MD, PhD, Jian Guo, MD, PhD

Cardiovascular disease is the leading cause of death worldwide, and accumulating evidence has demonstrated that mutations in the gene cluster *APOA5/A4/C3/A1* are associated with hypertriglyceridemia, which may predispose to atherosclerosis and cardiovascular morbidity and mortality (1–3). Apolipoprotein A5 (APOA5) is located proximal to this apolipoprotein gene cluster on chromosome 11q23 (RefSeq: NM_052968). Early animal studies with transgenic mice expressing a human *APOA5* transgene showed a decrease in plasma triglyceride concentrations, and knockout mice lacking APOA5 had a 4-fold increase in plasma triglyceride levels compared with controls (4). APOA5 is a component of several lipoprotein fractions including very low density lipoprotein, high-density lipoprotein, and chylomicrons, and it is believed that APOA5 affects lipoprotein metabolism by interacting with the low-density lipoprotein receptor family of gene receptors (5). Nonetheless, the precise mechanism by which APOA5 affects triglyceride levels and how triglyceride levels correlate with coronary vascular disease remains controversial (6). Current evidence points to a role for APOA5 in augmenting the triglyceride lipolytic process by stimulating lipoprotein lipase (7).

Epigenetic regulation, which has traditionally been known to include processes such as DNA methylation and histone modification has recently been found to involve the functions of microRNAs (miRNAs). These are single-stranded RNA molecules—21 to 23 nucleotides in length—that can bind the 3′ UTR region of messenger RNA transcripts to regulate gene expression by inhibition of protein synthesis, degradation of the messenger RNA, or both (8).

SEE PAGE 267

In this issue of the *Journal*, Cui et al. (9) present an innovative study that combines an epidemiological investigation on a large number of human subjects and an extensive evaluation of miRNA biology. These authors sequenced all the exons expressed from the *APOA5/A4/C3/A1* gene cluster in 200 individuals with extremely high triglyceride levels and 200 matched control subjects. They also genotyped 20 genetic markers among 4,991 participants of Chinese Han ethnicity. Finally, they identified a unique single nucleotide polymorphism (SNP) (rs2266788) located in the 3′ UTR region of the APOA5 transcript, which changes a single nucleotide from a T to a C. This SNP had a robust correlation with elevated plasma triglyceride levels and vascular lesions. This group also used approaches used by others to validate miRNA gene targets (10,11), including in vitro gain- and loss-of-function approaches that demonstrated that APOA5 with a T in the 3′ UTR position bound miR-3021, whereas the T to C SNP destroyed the binding and abolished the repression of *APOA5* gene expression by miRNA-3201. This report is highly novel, because little is currently known about miRNA-3201 (12). This study may stimulate future intensive investigations about the function of this SNP (rs2266788) and its related miRNAs, which will further unveil the underlying molecular mechanism associated with genomic variances in the *APOA5/A4/C3/A1* gene cluster, the epigenetic functions of regulatory miRNAs,

*Editorials published in the *Journal of the American College of Cardiology* reflect the views of the authors and do not necessarily represent the views of *JACC* or the American College of Cardiology.

From the Division of Cardiovascular Surgery and Toronto General Research Institute, University Health Network and Department of Surgery, Division of Cardiac Surgery, University of Toronto, Toronto, Ontario, Canada. Both authors have reported that they have no relationships relevant to the contents of this paper to disclose.

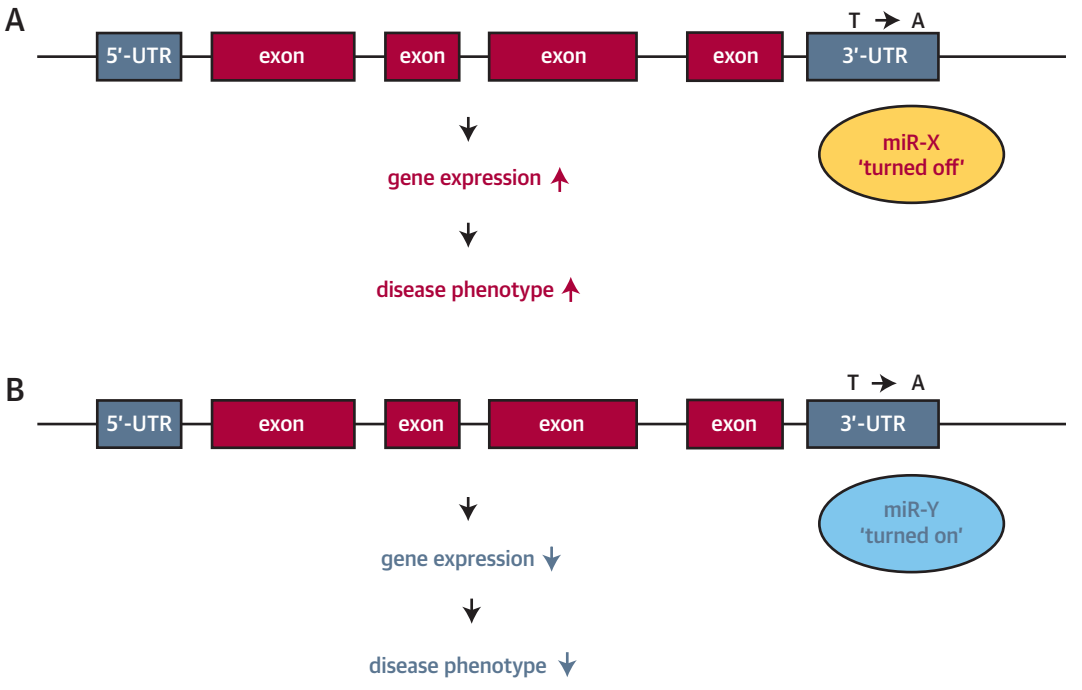

FIGURE 1 Cross Talk Between Genomics and Epigenetics

When a single nucleotide polymorphism occurs in the 3′ UTR region of a gene's transcript at a site where microRNAs bind and exert their regulation on gene expression associated with a particular disease, multiple distinct scenarios are possible. **(A)** In this example, a T- to-A change in the 3′ UTR of a gene abolishes the binding site of a microRNA (miR-X) that functions to repress this gene, resulting in increased gene expression and a more pronounced disease phenotype. **(B)** Yet the same T-to-A change also may create ≥1 more previously nonexisting binding site(s) for other microRNAs (miR-Y), resulting in decreased gene expression and alleviating the disease phenotype.

and the hypertriglyceridemia associated with this gene cluster, building a comprehensive foundation for future therapeutic interventions.

However, we must be aware that the nature of miRNA biology is extremely complex. Single miRNA does not simply regulate a single gene in a "one-to-one" fashion, but instead often functions in a "one-to-many" or "many-to-one" manner. The regulation of the ~25,000 genes in the human genome by the ~1,000 known miRNAs is highly dynamic and is surely more complicated than suggested by our current understanding. For example, a recent report by Caussy et al. (13) demonstrated that the T version (rather than the C version) of the same SNP (rs2266788) recognized by Cui et al. (9) creates a functional binding site for a different miRNA, miR-485-5p, which can modulate *APOA5* gene expression. It would be interesting for future investigations to define the cellular and tissue source of the newly discovered miRNA-3201 and to correlate the spatial and temporal regulation of miRNA-3201 expression levels and plasma triglyceride levels at different stages of the development of vascular disease in a tightly controlled animal model. In addition, future studies will be required to compare the interrelationship between miRNA-3201, miRNA-485-5p, and other microRNAs that could potentially regulate *APOA5* gene expression, triglyceride level in blood, and the occurrence of atherosclerosis. These studies eventually may translate our knowledge about this complex gene regulation into a potential novel therapeutic regimen.

SNPs often are located in noncoding genomic regions and are a major source of diversity among individuals. SNPs occurring in coding regions can result in mutant protein products, potentially causing protein dysfunction and genetic disease. However, SNPs located in the 3′ UTR region (**Fig. 1**) can illegitimately generate an artificial binding site or destroy a preexisting miRNA binding site. One such an example was recently reported: an obesity-associated SNP (rs8887) that creates an illegitimate miRNA-522 binding site in the 3′ UTR of the *PLIN4* gene and promotes down-regulation in adipose tissue (14). A plethora of similar studies associated with other

JACC VOL. 64, NO. 3, 2014
JULY 22, 2014:278–80

pathological conditions within the past 2 years has been reported (15–19). We are witnessing an emerging cross talk between functional genomics and regulatory epigenetics that may permit us to better understand and further investigate the complexity of the human genome.

REPRINT REQUESTS AND CORRESPONDENCE: Dr. Ren-Ke Li, MaRS Centre, Toronto Medical Discovery Tower, Room 3-702, 101 College Street, Toronto, Ontario M5G 1L7, Canada. E-mail: renkeli@uhnres.utoronto.ca.

REFERENCES

1. Priore Oliva C, Pisciotta L, Li Volti G, et al. Inherited apolipoprotein A-V deficiency in severe hypertriglyceridemia. Arterioscler Thromb Vasc Biol 2005;25:411–7.

2. Yamada Y, Matsuo H, Warita S, et al. Prediction of genetic risk for dyslipidemia. Genomics 2007;90:551–8.

3. Mendoza-Barberá E, Julve J, Nilsson SK, et al. Structural and functional analysis of APOA5 mutations identified in patients with severe hypertriglyceridemia. J Lipid Res 2013;54:649–61.

4. Pennacchio LA, Olivier M, Hubacek JA, et al. An apolipoprotein influencing triglycerides in humans and mice revealed by comparative sequencing. Science 2001;294:169–73.

5. Nilsson SK, Christensen S, Raarup MK, et al. Endocytosis of apolipoprotein A-V by members of the low density lipoprotein receptor and the VPS10p domain receptor families. J Biol Chem 2008;283:25920–7.

6. Hulley SB, Rosenman RH, Bawol RD, et al. Epidemiology as a guide to clinical decisions. The association between triglyceride and coronary heart disease. N Engl J Med 1980;302:1383–9.

7. Merkel M, Loeffler B, Kluger M, et al. Apolipoprotein AV accelerates plasma hydrolysis of triglyceride-rich lipoproteins by interaction with proteoglycan-bound lipoprotein lipase. J Biol Chem 2005;280:21553–60.

8. Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, and function. Cell 2004;116:281–97.

9. Cui G, Li Z, Li R, et al. A functional variant in *APOA5/A4/C3/A1* gene cluster contributes to elevated triglycerides and the severity of CAD by interfering with microRNA 3201 binding efficiency. J Am Coll Cardiol 2014;64:267–77.

10. Li S-H, Guo J, Wu J, et al. miR-17 targets tissue inhibitor of metalloproteinase 1 and 2 to modulate cardiac matrix remodeling. FASEB J 2013;27:4254–65.

11. Wang Y-S, Li S-H, Guo J, et al. Role of miR-145 in cardiac myofibroblast differentiation. J Mol Cell Cardiol 2014;66:94–105.

12. Stark MS, Tyagi S, Nancarrow DJ, et al. Characterization of the Melanoma miRNAome by Deep Sequencing. PloS One 2010;5:e9685.

13. Caussy C, Charrière S, Marçais C, et al. An APOA5 3′ UTR variant associated with plasma triglycerides triggers APOA5 downregulation by creating a functional miR-485-5p binding site. Am J Hum Genet 2014;94:129–34.

14. Richardson K, Louie-Gao Q, Arnett DK, et al. The PLIN4 variant rs8887 modulates obesity related phenotypes in humans through creation of a novel miR-522 seed site. PloS One 2011;6:e17944.

15. Liu M-E, Liao Y-C, Lin R-T, et al. A functional polymorphism of PON1 interferes with microRNA binding to increase the risk of ischemic stroke and carotid atherosclerosis. Atherosclerosis 2013;228:161–7.

16. Cheng M, Yang L, Yang R, et al. A microRNA-135a/b binding polymorphism in CD133 confers decreased risk and favorable prognosis of lung cancer in Chinese by reducing CD133 expression. Carcinogenesis 2013;34:2292–9.

17. Gallego X, Cox RJ, Laughlin JR, et al. Alternative CHRNB4 3′-UTRs mediate the allelic effects of SNP rs1948 on gene expression. PloS One 2013;8:e63699.

18. Xu Y, Ma H, Yu H, et al. The miR-184 binding-site rs8126 T>C polymorphism in TNFAIP2 is associated with risk of gastric cancer. PloS One 2013;8:e64973.

19. Minguzzi S, Selcuklu SD, Spillane C, et al. An NTD-associated polymorphism in the 3′ UTR of MTHFD1L can affect disease risk by altering miRNA binding. Hum Mutat 2014;35:96–104.

KEY WORDS *APOA5/A4/C3/A1* gene cluster, coronary artery disease, genetics, lipids